THE EFFECT OF SEWAGE EFFLUENT AND DEICING SALT ON THE GREEN ALGA *SELENASTRUM CAPRICORNUTUM*

An abstract of a Thesis by
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The problem. This study was undertaken to evaluate the impact of secondary sewage effluent and highway deicing salt runoff on a planktonic green alga.

Procedure. The test organism, *Selenastrum capricornutum*, was grown in an algal assay which combined various concentrations of deicing salt and sewage effluent diluted with Des Moines River water. Monthly river and sewage samples were taken from November 1976-March 1977. Growth response was measured with a chlorophyll assay.

Findings. Stronger sewage concentrations gave greater responses. The addition of 5,000 mg Cl/l was stimulating in any combination of river water and sewage effluent. It appeared that 10,000 mg Cl/l, while not entirely inhibitory, was slightly toxic since the growth response was less than the lower salt concentration and more like that of straight river water. Statistical analyses indicated that fluctuations in river water composition occurred over time since monthly growth responses differed significantly from each other. November samples gave the lowest chlorophyll values and collections from March usually had the greatest which corresponded to nutrient values determined by chemical analyses of the samples.

Conclusions. The addition of both sewage effluent and deicing salt to Des Moines River water had a growth-enhancing effect on the test alga, *Selenastrum capricornutum*, in most combinations.

Recommendations. Further study should include a year-round survey with more sampling sites and frequent collections to determine present chloride levels and serve as a basis for predicting future trends. Experiments dealing with nutrient spikes ought to be considered. The use of indigenous algal species and *in situ* observations would be valuable.

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A Thesis

Presented to

The School of Graduate Studies

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by

Susan Rebecca Arneson

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INTRODUCTION

The quality of existing water supplies is becoming a major concern for most Americans today. Drought has plagued many areas of the country, recently California and the upper Midwest. Groundwater is being rapidly consumed and slowly replaced. Consequently, water tables are dropping and streams and rivers are experiencing their lowest flows in years, making surface water an even more important commodity. With shortages increasing, it is of utmost importance that the quality of water be maintained, preserved, and improved where possible.

Low stream flows bring the added problem of limited dilution capabilities where it is often needed since streams are a convenient disposal area for municipal wastewater effluents. As a result, aquatic biota have been subjected to severe changes in their environment. This has caused the displacement of many naturally occurring organisms and the predominance of less desirable species which seem more tolerant of new environmental conditions.

The problem of wastewater disposal and associated environmental effects has been observed by many researchers. Investigations have been designed to correlate the ecological condition of surface waters to the composition and abundance of aquatic populations. A common objective is the evaluation of the significance of indigenous species as indicators of ecological changes produced in the stream by changing water quality.

Lackey (1956) reported increased numbers of organisms, frequent algal blooms and changes in the dominant species downstream from a sewage treatment plant on Lytle Creek, Ohio. A classic example of the before and after effects of sewage effluent diversion around several lakes in Madison, Wisconsin, was documented by Mackenthun et al. (1960) and Wisniewski (1961).

Results from Patrick's (1966) experiments indicated that factors such as organic substances or trace elements associated with wastewater effluent may be important, probably in association with increased nitrogen and phosphorus, in producing large diatom growths. Based on Lake Washington observations of sewage enrichment and diversion, Edmondson (1972) suggested that predictions of effects of nutrient additions should not be based on measurements of a single nutrient due to interactions that occur.

The North Saskatchewan River provided a unique study for Paterson and Nursall (1975). Municipal and industrial effluents entered the south shore and remained unmixed with water on the north side due to current speed and river width. Chemical parameters measuring higher along the south side were combined with a general biomass increase through that region with marked changes in the biota.

The use of *in situ* algal assays has been valuable in assessing the algal growth potential of surface waters with known nutrient additions by observation of the natural phytoplankton community response. Field experiments were performed by Powers et al. (1972) in Minnesota and Oregon. The Oregon experiments involving nutrient enrichment of lakes with varying productivity levels indicated that while phosphorus was initially limiting for algal growth, carbon and nitrogen became limiting when sufficient phosphorus was present although exact results depended on lake trophic level. Controlled phosphorus input to eutrophic Shagawa Lake, Minnesota, was considered the most likely way to establish lake recovery.

Megard (1969) measured the effects of different amounts of effluent from secondary and tertiary sewage treatment plants on algae. Algal photosynthetic capacity was stimulated by 5% and 10% secondary effluent, inhibited by 20% secondary effluent and tertiary effluent only slightly stimulated photosynthesis.

Bahls (1973), studying the response of the diatom community in the East Gallatin River, Montana, to the addition of primary wastewater effluent, indicated species diversity had a negative correlation with ammonia and a positive correlation with phosphate.

Chemical analysis of water gives information on the quantity of plant nutrients present, but supplies no knowledge of their availability for algae. The algal assay involves a single algal species subjected to changes in one or several factors under controlled laboratory conditions. Results may determine some biological effect, measure the concentration of a substance or indicate the nature of the physical environmental conditions. In recent years, assays have been developed which form a basis for comparison of results between labs or from different samples (Weber, 1973a).

Meffert (1955) attempted to cultivate a green alga in sewage as a means of wastewater purification and possible harvest for animal feed. A green alga cultured by Dor (1975) was grown in dialysis tubing suspended in sewage. Both concluded that the amount of dissolved CO_2 seemed to limit algal growth response in the presence of abundant nutrients.

Skulberg (1967) grew *Selenastrum capricornutum* in controlled but varying nutrient levels and showed a direct correlation with nutrient amounts and algal yields. Algal assays were used by Miller and Maloney (1971) to determine and predict the effects of secondary- and tertiary-treated waste effluent on algal growth in Shagawa Lake and Burntside River (Minnesota) water. Algal growth responded positively to the addition of secondary effluent to both waters. Tertiary wastewater effluent would not support the growth of the test alga unless phosphorus (even in minute quantities) was added despite the presence of all other nutrients.

McDonald and Clesceri (1973), studying organic fractions separated from wastewater effluent, found the fractions exerted a growth-enhancing effect on algal assays. A survey of the Snake River basin utilizing algal assays revealed phosphorus or nitrogen to be the algal growth-limiting nutrients in a majority of the waters tested (Greene et al., 1975).

Ferris et al. (1974) concluded that secondary sewage, with or without detergent phosphorus, could be a major source of nutrients and enhanced the growth of *Selenastrum capricornutum* (Printz) to various degrees in all lake waters tested with tertiary treatment to remove phosphorus successful in decreasing the algal response. Schelske et al. (1974) reported increased standing algal crop with increased phosphorus although noting a greater response to high phosphorus levels than would be represented by similar increases in relative abundance in low phosphorus treatments. Results of tests with *S. capricornutum* by Shiroyama et al. (1975) indicated that: (1) major phosphorus and nitrogen uptake occurred in the first 5 days of growth; (2) a higher growth rate was observed with phosphorus as compared to nitrogen; (3) when nitrogen was not growth-limiting, maximum yield increased in proportion to each phosphorus addition; and (4) there was a definite linear relationship between biomass produced and the amount of phosphorus and nitrogen present.

Emery et al. (1973) used algal assays to determine that the nutrient contribution of urban surface water runoff to the eutrophication level of a lake was not significant. Sartor et al. (1974), however, contended that storm runoff from urban areas was an important source of pollutants and urged studies to measure amounts involved and to establish possible control.

Another area water quality experts are dealing with encompasses environmental damage caused by street deicing salts, usually monitored by tracing the chloride ion. The U.S. Public Health Service has set the chloride concentration limit at 250 milligrams per liter (mg/l) allowed in water used for public consumption. William E. Dickinson, President of the Salt Institute, expressed his concern in an interview (1971) that legislation may restrict the use of chlorides. He stressed that salt is the most effective and least expensive safety aid the highway maintenance engineer has to work with, though he agreed that better control of application and storage is needed.

Lockwood (1965) and Keyser (1973) both reviewed the literature involving deicing chemicals and abrasives used for winter road maintenance. Sodium chloride ($NaCl$) is generally used at temperatures above 10°F(-12°C) and is much more economical than $CaCl_2$. Calcium chloride ($CaCl_2$) is most effective at temperatures above -30°F (-34°C), so mixtures of the two salts are often used in conjunction with abrasives. The Public Works Administration of Des Moines, Iowa (personal communication), uses a majority of rock salt in its deicing program.

Many natural or manmade lakes receive runoff water directly from highways or storm sewers and are closed basins so salt concentrations increase rapidly. The major physical effect is due to more dense saline water moving to deep parts of the lake basin and remaining separate from lower density fresh water.

Studies by Bubeck et al. (1971) and Diment et al. (1973) indicated that chloride concentrations in Irondequoit Bay, which receives runoff from Rochester, New York, have risen 10 times since 1910. They pointed to adoption of the bare pavement policy and unrestrained use of deicing salt as creating a density stratification sufficient enough to delay fall mixing of the bay by one month.

Runoff from street deicing carried water of greater density into First Sister Lake near Ann Arbor, Michigan, and appeared to be the major factor inhibiting spring mixing (Judd, 1970). There was also an indication of ion movement into the lake sediments, with the possibility that the salt further leached into the groundwater.

Cherkauer and Ostenso (1976) monitored chloride levels in three artificial lakes near Milwaukee, Wisconsin. Mean chloride concentrations of outflow during runoff periods varied from a low of 84 mg/l in November 1975 after a high of 270 mg/l in March 1975, with a July 1975 value of 243 mg/l which indicate that the lakes provided a storage site for dissolved salts year round.

Street salt application can contribute to measurable increases in chloride concentrations but relatively weak influences on the content of large lakes and rivers. Major impairment of surface water quality seems to be temporary due to gradual dilution of the salt by later precipitation. However, rising trends in chloride concentrations have been reported.

A survey of Wisconsin's waters by Schraufnagel (1967) showed that highest chloride concentrations occurred in southeastern Wisconsin and were directly proportional to population and number of highways in the area. Chloride levels of 1,510 to 2,730 mg/l found in the Milwaukee, Menomonee, and Kinnickinnic Rivers at Milwaukee, Wisconsin in January 1969 were attributed by Field et al. (1973) to deicing salts entering these streams from highway snow melt.

An Environmental Protection Agency (1971b) report cites the results of street runoff samples collected from a downtown Chicago, Illinois, expressway during the winter of 1967. When highway salts were not being applied, the chloride content of drainage varied from 1,900 to 4,500 mg/l, while during snowfalls, it ranged from 11,000 to 25,000 mg/l. Walker (1971) noted a persistent yearly increase in the chloride levels of the Illinois River at Peoria which paralleled the rock salt use for snow and ice control within the state. By following the movement of chloride in the

Salt Creek basin in the Northeastern Illinois Metropolitan Area, Wulkowicz and Saleem (1974) found that approximately 73% of the chloride was solubilized and removed by the creek waters.

The Environmental Protection Agency (1971b) cited a study of the Des Moines River and Raccoon River above and below Des Moines, Iowa, which concluded that chloride levels were significantly higher in the winter months than in the summer. Downstream from Des Moines, chlorides often exceeded 56 mg/l with a maximum level of 86 mg/l in January and February 1969. During the spring and summer months, chloride concentrations were below 20 mg/l, but as stream flow diminished in the autumn, the chlorides increased to between 20-30 mg/l.

Meadow Brook in Syracuse, New York, usually contained chloride concentrations in the range of 200 to 1,000 mg/l, but frequently exceeded several thousand mg/l in data gathered by Hawkins and Judd (1972). The chloride content of the watershed reached a high of about 11,000 mg/l in December 1969.

The Ontario Water Resources Commission (1971) and Van Loon (1972) observed that chloride inputs from road salting operations in the metropolitan Toronto area significantly increased concentrations from 105 mg/l to 452 mg/l in the Don River. Sewage plant effluent also showed increases in chlorides from 266 mg/l to 3,100 mg/l during salt runoff.

Scott (1976) sampled Black Creek, which flows through Toronto to Lake Ontario, in an effort to determine the pattern of salt movement during a major thaw. His data indicated that salt runoff occurs in waves dependent on temperature, with high concentrations rapidly diluted by snow melt, though they may accumulate in lakes further downstream. Using a regression equation, he calculated chloride values based on observed sodium concentrations which ranged from a low of 250 mg/l in December to 3,787 mg/l after a January thaw.

Temporarily high chloride concentrations in surface waters near Louvain, Belgium, were not considered to be a problem by Van de Voorde et al. (1973) as compared to the benefits of traffic safety provided by deicing practices. The highest chloride value of 674 mg/l occurred in a stream with low water flow but returned to normal fluctuating levels seven days after the last salt scattering.

Road salt investigations in the Sleepers River (Vermont) basin (Kunkle, 1972) revealed that chloride values in the highway-influenced stream peaked during summer baseflow to approximately 62 mg/l in August. The lowest chloride levels, about 5 mg/l during the spring snowmelt period, were thought to be due to large quantities of dilution water. High summer concentrations indicated that some road salt percolated through soils to subsurface flows and emerged in summer's groundwater inputs into the stream. Individual seeps sampled near the highway showed chloride levels exceeding 200 mg/l.

Hutchinson (1968) concluded that highway salting had no strong influence on the concentration of chloride in the Maine rivers sampled. However, in a later project, Hutchinson (1969) noted that chloride contamination of private wells followed a close relationship with proximity to salted roads (concentrations decreased with distance from the highway). Huling and Hollocher (1972) suggested that if present rates of salt application continue in the suburban Boston area, the current average steady-state chloride concentration in groundwater should approximate 100 mg/l.

Groundwater has been affected measurably by road salt chloride, and the duration of water quality impairment is considerably longer than it is for streams. Pollock and Toler (1973) investigated chloride water supply contamination in Massachusetts and noted that in 1961 water from a well located near two major highways had a chloride concentration of less than 15 mg/l. Uncovered salt storage and liberal salt use resulted in chloride levels that exceeded 250 mg/l by 1970.

Walker and Wood (1973) suggested that the base level chloride concentrations generally reflected the salt pollution level of groundwater outflow to a stream. Groundwater pollution occurs with precipitation recharge from poorly protected salt piles or heavily salted roads after the ground thaws.

Research dealing directly with the effects of salt on algae has been sparse. Hanes et al. (1970) noted that studies oriented toward evaluating the specific effects of salts have been limited primarily to plant compositional changes brought about by introducing a salt into a solution with a constant nutrient content. The toxic effect of chloride ion accumulation has often been named as causing direct damage to the plant.

Vosjan and Siezen (1968), using two species of green algae, showed that the marine alga could stand greater variations in salinity than the freshwater alga. Both had an optimum photosynthetic rate at salinities nearest those found in their natural environments.

Chimiklis and Karlander (1973) grew Chlorella in various NaCl concentrations and noted decreased growth with greater concentrations. However, the addition of small amounts of calcium extended the tolerance and, therefore, the growth limits of the green alga.

Pollutants in Iowa waters include: (1) silt from agricultural runoff, (2) nutrients from agricultural non-point and point sources such as fertilizers and feedlots, and (3) sewage enrichment. Turbidity and suspended solids cause the major physical modification problems in the Des Moines River and have a strong influence on algal populations (Iowa Department of Environmental Quality, 1975). Non-point sources are the major contributors of phosphates and nitrogen compounds to all state surface waters. Nutrients are present in high concentrations directly related to runoff conditions. Jones and Bachmann (1975) found a direct correlation between algal growth and nutrient input, in the form of nitrogen and phosphorus, in the northwestern lakes of Iowa.

The nutrient and suspended algae levels in central Iowa streams were examined by Kilkus et al. (1975). Nutrient levels, with surface runoff as the major contributor, are so high that nitrogen and phosphorus are not limiting to phytoplankton growth which seems to be held in check by physical factors (i.e., turbidity, light penetration, temperature, flow). Surface runoff and combined sewer overflows can significantly raise the pollutant levels of an urban watercourse and are not to be neglected as important non-point discharges.

Approximately 96% of Iowa's incorporated population is served by some form of sewage treatment facility (Iowa DEQ, 1975). Federal requirements for 1977 insure that most, if not all, major industries in the state will also be providing the best practicable treatment of their wastewater. Tertiary treatment of sewage is recommended by Baumann and Kelman (1970) to remove nitrogen and phosphorus from wastewater and thus decrease algal growth in receiving waters.

They indicated that nutrient sources of the Des Moines River vary during periods of low and high river flow from easily controlled waste discharges to non-point sources such as agricultural runoff which are difficult to monitor.

Chloride levels have not warranted serious consideration in most Iowa surface waters though they represent an important concern in some. The Big Sioux River boasts the highest chloride concentrations in the state with an average of 125 mg/l and maximums well over 150 mg/l (Iowa DEQ, 1975). Another northwestern Iowa river, the Floyd, also shows consistently elevated chloride levels. The Maquoketa River receives large volumes of saline waste from hide curing operations in Manchester which significantly raise chloride amounts.

The average concentration for the Des Moines River is approximately 28 mg/l (Iowa DEQ, 1975), although chlorides appear to be increasing. Day (1972) indicated that deicing salt did not affect the chloride concentration of the Des Moines River. His chloride values averaged from 20-30 mg/l at the three stations sampled in Des Moines during the winter of 1970-71. An environmental impact statement of a proposed freeway around southeastern Des Moines (Merkley, personal communication) suggested that little ecological damage will result from winter maintenance. Although the chloride concentration may range from 350-500 mg/l or greater at the time of salting, he speculated that such high concentrations would be short-lived due to dilution.

From the literature surveyed, it is evident that there has been little emphasis on the ecological effects of deicing programs on the aquatic environment. The general conclusion by researchers is that most of the salt runoff reaches local surface waters. These waters usually receive effluents from municipal and industrial wastewater treatment plants as well. It is the combined effect of these two factors that is of interest in this project.

This study was undertaken to evaluate the impact of sewage effluent and highway deicing salt runoff on a planktonic green alga grown in water collected from the Des Moines River. The objectives of the assay were to determine:

(1) the algal growth-supporting capabilities of the Des Moines River

(2) the effect of domestic secondary sewage effluent on the growth of the test organism

(3) the effect of highway deicing salt runoff on the growth of the test organism

(4) the stimulatory and/or limiting effects of combined sewage effluent and salt runoff

(5) if the effects change with varying concentrations and combinations of each factor.

MATERIALS AND METHODS

Des Moines River water (RW) was obtained near the Southeast 14th Street Bridge upstream from its confluence with the sewage treatment plant (STP) effluent. The sampling site for the sewage effluent (SE) was the outfall to the river from the final clarifiers at the Des Moines Municipal STP. River water and sewage effluent used for each experiment were collected the same day and returned to the laboratory for immediate chemical analysis. Tests conducted in the field were temperature, free CO_2, and alkalinity (Welch, 1948). The chemical tests for pH, specific conductance, orthophosphate, nitrite-nitrogen, nitrate-nitrogen, and chloride were performed in the laboratory within two hours of collection. Specific conductance was determined with a Beckman Solu Bridge and pH with a Coleman Metrion III pH meter. Nitrite-nitrogen was analyzed by the diazotization method and nitrate-nitrogen by the cadmium reduction method (Hach Chemical Company, 1975). Dissolved inorganic chlorides were measured by the argentometric method and orthophosphates by the stannous chloride method (APHA, 1975).

Upon completion of the chemical tests, the remaining water samples were autoclaved at 15 psi at 121°C for 30 minutes to solubilize nutrients in the indigenous biomass. After autoclaving, the samples were allowed to cool and equilibrate to restore CO_2 lost during autoclaving and to return pH to the original level. The autoclaved sample was then passed through a Whatman No. 7 filter paper to remove large particulates and finally through a 0.45 micron Millipore filter to remove any remaining debris. Prepared water samples were stored in the dark at 4°C in acid-rinsed glassware.

Selenastrum capricornutum (Printz), recommended for use with the Algal Assay Procedure (AAP): Bottle Test (EPA, 1971a), was the test alga used in this study. Taxonomically, it belongs in the Chlorophyceae (green algae), Order Chlorococcales, Family Selenastraceae. S. capricornutum has the advantage of being easy to count as it is typically unicellular or aggregated into small groups and maintains a uniform crescent-moon shape with varying nutrient conditions. It is characteristically tolerant of organic pollution and is the indicator organism of choice in many current algal assay studies. A stock culture (#1648) was obtained from Dr. Richard C. Starr, curator of the Culture Collection of Algae at the University of Texas and maintained according to the specifications in the AAP (EPA, 1971a). This stock culture served as the source of inoculum for each experiment. Modifications to the published procedure were necessary to maintain the growth chamber (Lab-Line Biotronette Mark III Environmental Chamber) at a more constant and optimal temperature of 26° ± 2°C. The illumination was kept at 97.5 foot-candles for 18 hours alternating with 6 hours of darkness.

The inoculum was prepared by concentrating the stock culture in an International Refrigerated Centrifuge Model PR-2, at 1000 g, discarding the supernatant, and resuspending the algal cells in a 0.0002 M $NaHCO_3$ solution. This procedure was repeated several times for each inoculum to obtain rinsed concentrated cell suspensions. Cell concentration was determined by a direct cell count using a hemacytometer. The number of cells in each corner grid was counted, grids averaged, and the means multiplied by 10 to give the number of cells per milliliter. The appropriate volume of inoculum was then added to each flask to give a final cell concentration of approximately 1000 cells per milliliter.

Water samples were collected at regular intervals (about once every 4 weeks) from November 1976 through March 1977. To satisfy the criteria for a 3 x 3 factorial experimental design, nine solutions were mixed for each experiment. They included: (1) straight river water (RW), no sewage effluent (SE), no salt; (2) 2 parts RW, 1 part SE, no salt; (3) 1 part RW, 2 parts SE, no salt; (4) straight RW, no SE, 5,000 mg Cl/l; (5) 2 parts RW, 1 part SE, 5,000 mg Cl/l; (6) 1 part RW, 2 parts SE, 5,000 mg Cl/l; (7) straight RW, no SE, 10,000 mg Cl/l; (8) 2 parts RW, 1 part SE, 10,000 mg Cl/l; (9) 1 part RW, 2 parts SE, 10,000 mg Cl/l. The chloride levels of the first three solutions (those without salt) were determined and enough rock salt was added to raise the chloride levels of the salt-containing solutions to the needed concentrations. The rock salt used was a mined salt spread by highway maintenance crews to keep winter roads clear. Because it was not 100% pure, slight variations in the chloride concentration of the test solutions occurred. Triplicate replicates were prepared so that a total of twenty-seven 250 ml Erlenmeyer flasks containing 60 milliliters of solution were inoculated and incubated for each experimental run.

The experimental treatments were selected so differences in algal growth response between mixtures would be large enough to be easily measured. Although the experimental chloride concentrations are much higher than the normal winter chloride values found in local surface waters, they are within the range of typical values for rivers near large municipalities in the northern snow belt, i.e.--Chippewa Falls, Wisconsin (Schraufnagel, 1967); Syracuse, New York (Hawkins and Judd, 1972); Rochester, New York (Diment et al., 1973); Milwaukee, Wisconsin (Field et al., 1973); Chicago, Illinois (Wulkowicz and Saleem, 1974).

A standard growth curve was prepared for the test alga (Selenastrum capricornutum) according to the AAP (EPA, 1971a) culture method with modifications for illumination and temperature (Figures 1 and 2). For the standard growth curve of the test organism, dry weight, ash-free dry weight, and direct cell counts (Weber, 1973b) were measured. A

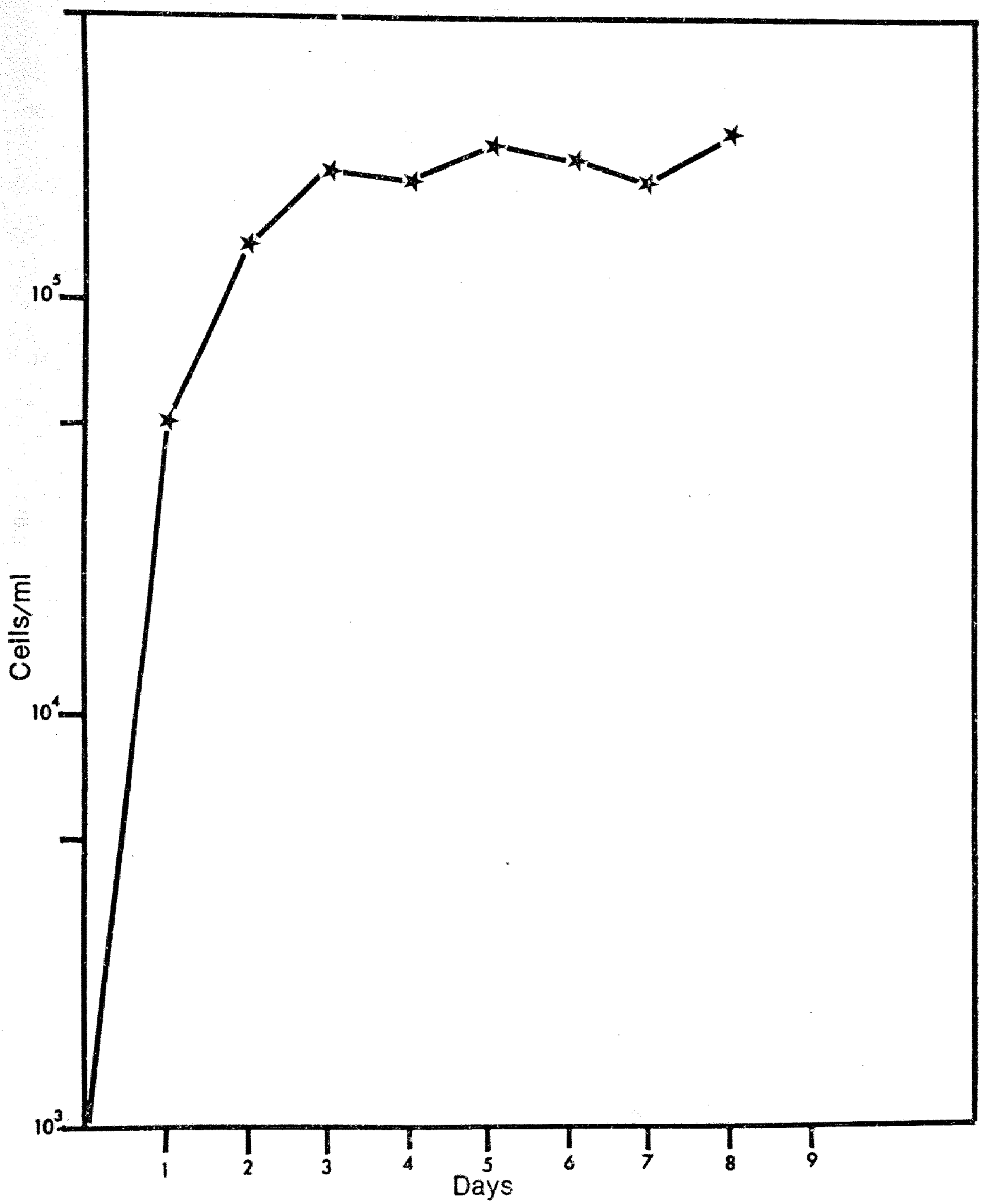

Figure 1. Standard growth curve of Selenastrum capricornutum at 26° ± 2°C, 97.5 foot-candles.

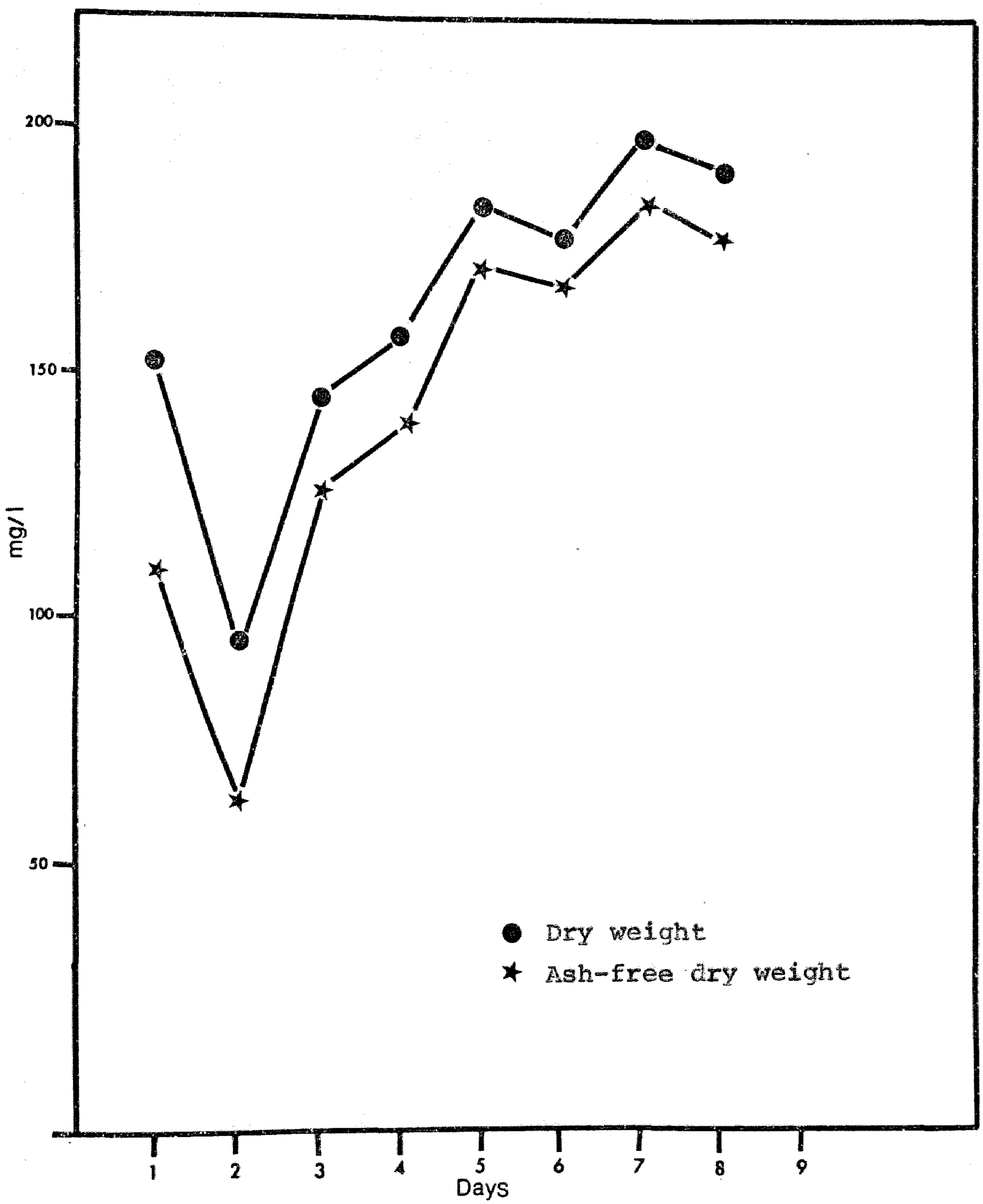

Figure 2. Standard growth curve of *Selenastrum capricornutum* at 26° ± 2°C, 97.5 foot-candles.

chlorophyll assay was also done but did not provide enough information to warrant its inclusion. Thirty-two flasks were initially inoculated with 1000 cells per milliliter. Each contained a total volume of 60 milliliters of complete growth media (EPA, 1971a). Every day for 8 days, 4 flasks were removed from the growth chamber. Dry weights and ash-free dry weights were determined from two flasks. Direct cell counts and a chlorophyll assay were performed on the remaining two. Graphs of the averaged results were prepared and the asymptote was found to occur on day 7.

The growth response of each experimental flask was measured after 7 days incubation by the trichromatic method for chlorophyll (Weber, 1973b). First 0.2 milliliter of a saturated aqueous magnesium carbonate solution was added to each flask. Next the contents were filtered through a Type A-E Gelman glass filter. The filter was then macerated in 2-3 milliliters of 90% acetone with a mechanical tissue grinder. The mixture was transferred to a conical centrifuge tube, capped, and stored in the dark at 4°C for at least 24 hours but not longer than 2 weeks before reading.

In preparation for spectrophotometric analysis, each sample was centrifuged for 20 minutes in a clinical centrifuge and the volume was measured by decanting the supernatant into a graduated cylinder. The chlorophyll assay was performed in a Perkin-Elmer Model Coleman 55 spectrophotometer at wavelengths of 750, 663, 645, and 630 nm. Sometimes dilution of the extract was necessary to bring the optical density at 663 nm to between 0.20 and 0.50 to minimize error. After the previous readings were taken, one drop of 1N HCl was added to each sample which was then reread at wavelengths of 750 and 663 nm. The calculations used to determine the amounts of chlorophylls a, b, c, and pheophytin a are found in Weber (1973b).

Measurements for chlorophyll a (mg/m^3 or μg/liter) were analyzed statistically by a factorial design using three factors--salt, sewage effluent, and time (Bruning and Kintz, 1977). This allowed evaluation of the main effects and interaction among the three variables. Results from the analysis indicated that the Newman-Keuls' multiple range test (Bruning and Kintz, 1977) could be used to determine which experimental conditions differed significantly from the others. The Pearson product-moment correlation analysis (Bruning and Kintz, 1977) was employed to determine the relationship between algal growth and nutrient (phosphate and total nitrite-nitrate) levels.

RESULTS

Figures 3, 4, and 5 record daily temperature, precipitation, and salting, respectively, for the test period of November 1976-March 1977. January 1977 had the coldest average temperature of the five months, the greatest amount of snowfall, and the most number of salting days (a total of

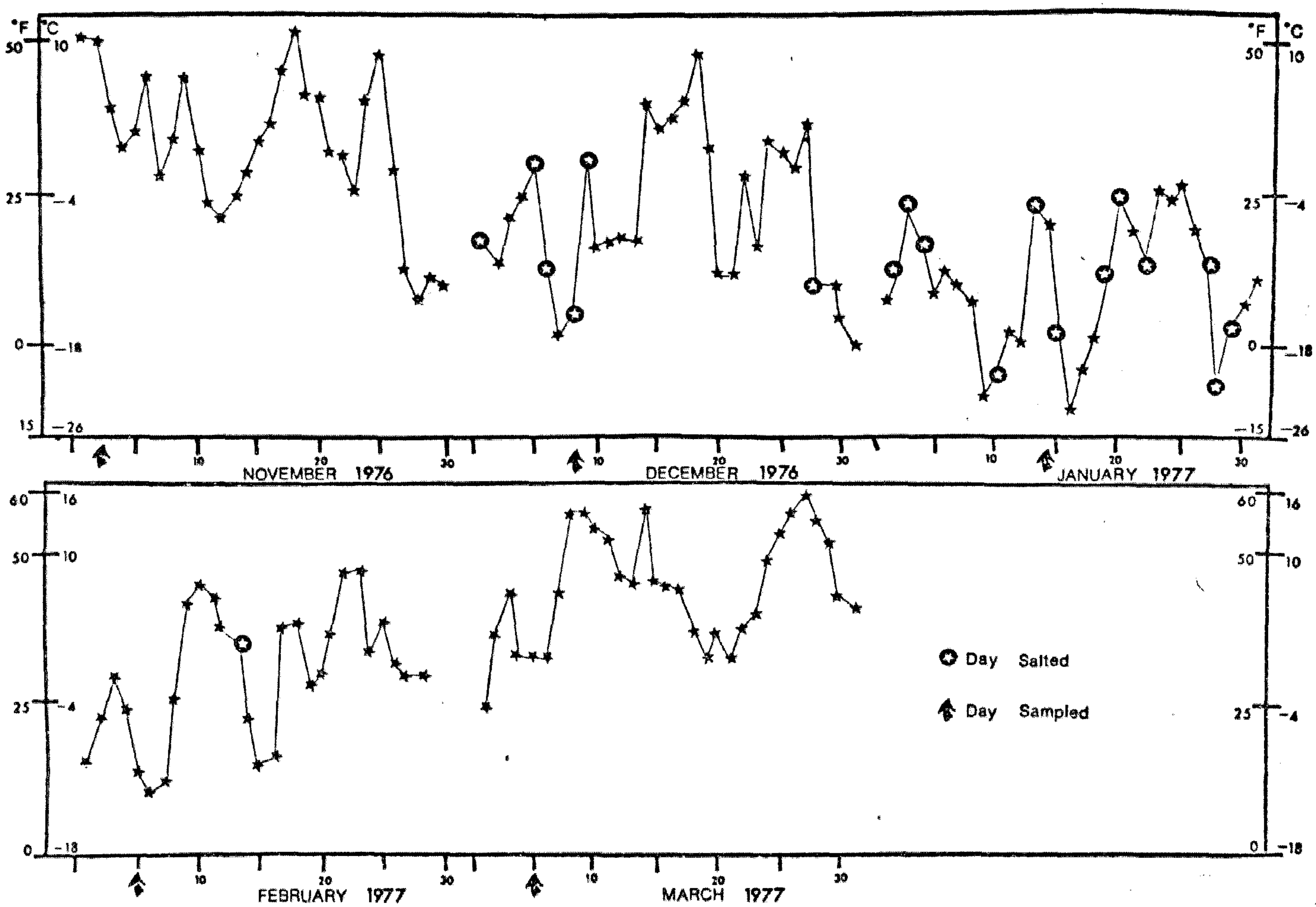

Figure 3. Average daily temperature for Des Moines, Iowa, November 1976-March 1977.

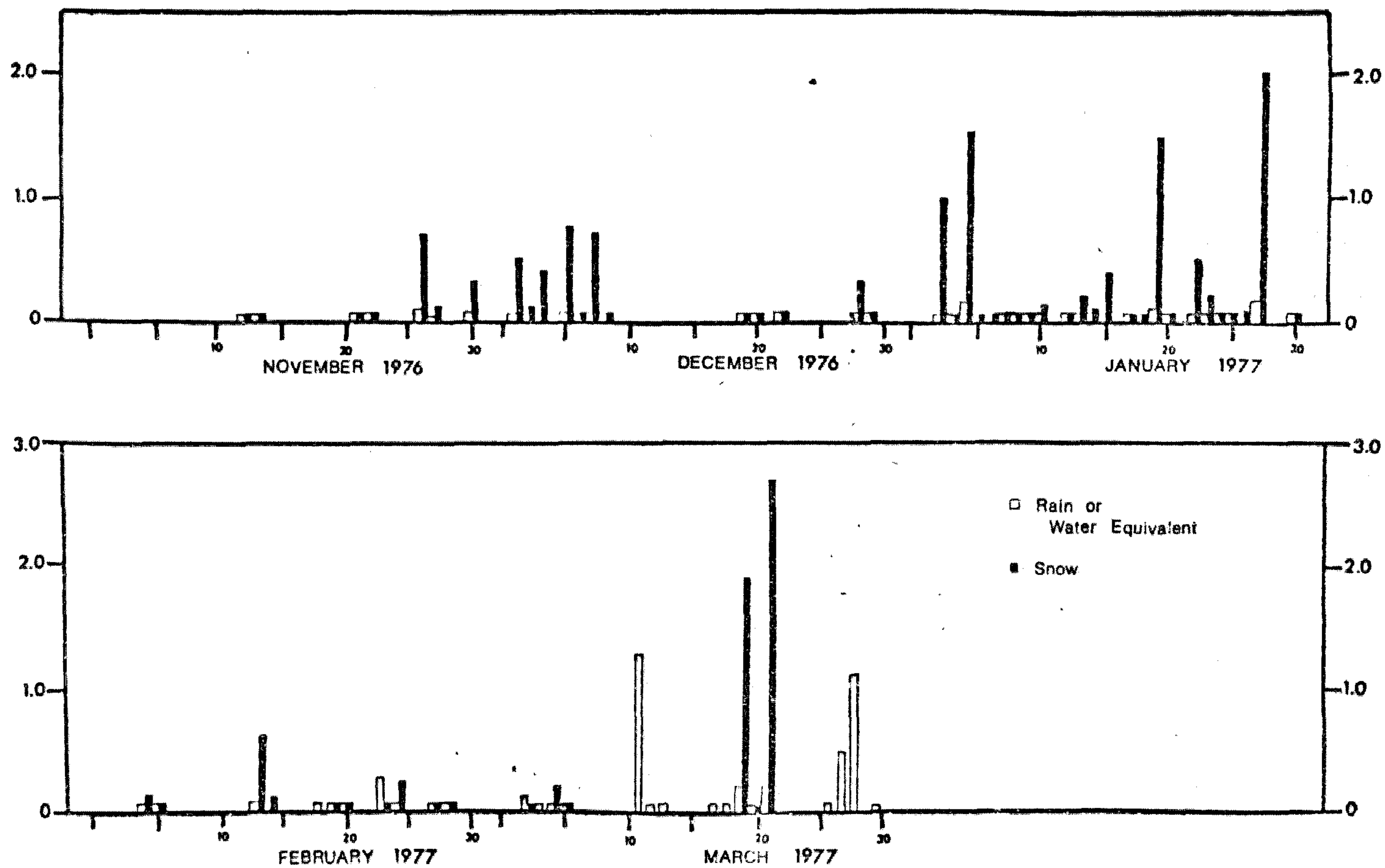

Figure 4. Daily precipitation (inches) for Des Moines, Iowa, November 1976-March 1977.

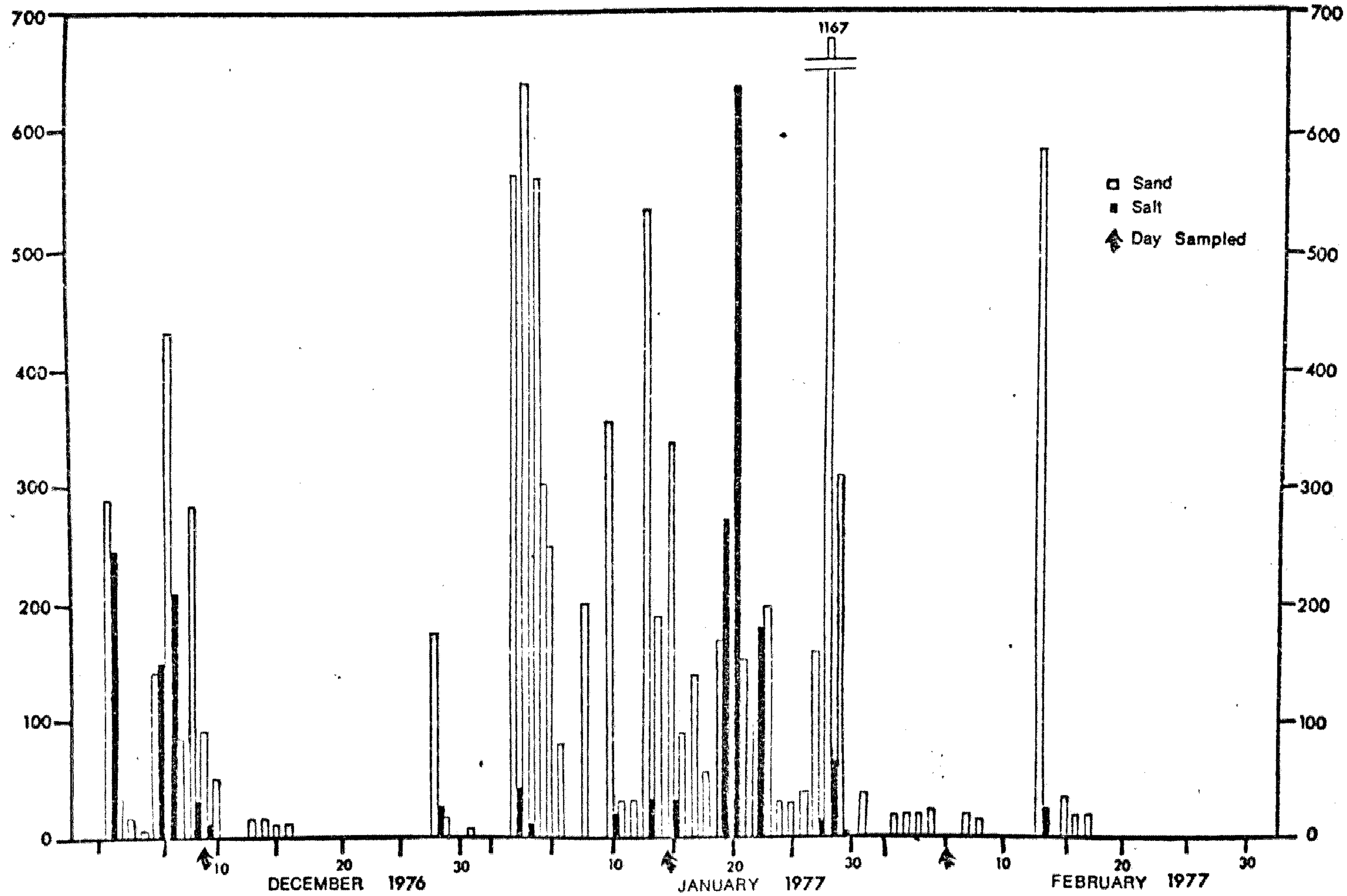

Figure 5. Salt and sand (tons) applied in Des Moines, Iowa, Winter 1976-1977.

12) resulting in an accumulation of deicing salt on city streets. Samples were taken January 14, one day after a temperature of -5°C (23°F). However, the average temperatures of the four previous days were less than -17.8°C (0°F), so snowmelt was slight and the chloride value moderate.

The temperature reached -4°C (25°F) on February 3 after six days of progressively warmer weather which allowed the snow to melt somewhat. When collections were made on February 5, much of the salt accumulation had entered the sewers and eventually, the Des Moines River, as runoff. Thus, the February chloride value was greater than any other month.

The March chloride concentration is the lowest of five months. By March 5, most of the snow had melted in the mild February weather (the average daily temperature was -1°C (30°F)) carrying away much of the road salt. Also, city maintenance crews spread less salt (only one day) in February. The fact that most of the runoff and dilution of the river chloride concentrations had already occurred contributed to the low March chloride value.

Table 1 lists nutrient values of river water and sewage effluent before (immediately after collection) and after sample preparation by filtering and autoclaving. In general, filtering tends to remove particulates with phosphates adsorbed to them and lowers the $P\text{-}PO_4$

Table 1. Phosphate, Total Nitrite-Nitrate, and Chloride values of samples (Des Moines River water and sewage effluent) before and after autoclaving and filtering, November 1976-March 1977. $P-PO_4$ - mg/l; N - mg/l; Cl - mg/l

		Before RW	After RW	Before SE	After SE
Nov 2	P	----	0.04	----	1.25
	N	0.20	0.52	3.17	4.52
	Cl	84	58	106	82
Dec 8	P	0.17	0.03	2.95	1.13
	N	1.73	1.70	2.62	3.34
	Cl	56	56	96	100
Jan 14	P	0.41	0.03	3.90	0.75
	N	0.72	1.26	2.16	2.23
	Cl	68	76	110	118
Feb 5	P	0.22	0.03	4.00	0.55
	N	1.63	1.38	4.76	2.70
	Cl	92	102	125	140
Mar 5	P	0.37	0.13	3.40	0.88
	N	1.13	1.47	3.53	3.34
	Cl	45	46	97	102

concentration. Autoclaving solubilizes organic material which releases nitrogenous compounds into solution and increases their amounts.

Chlorophyll a values calculated for each flask grown in the nine experimental solutions are listed by month in Table 2. The asterisk (*) by several chlorophyll a values in Table 2 denotes a value that is significantly different from the other two replicate flasks (EPA, 1971a) and is not plotted on Figures 6-11. The most likely reason for such an outlier is an uneven inoculation of the flask (with too few or too many algal cells). Each value was included, however, in the statistical analysis (Bruning and Kintz, 1977). Since river water and sewage effluent would naturally have a slightly variable composition, such variations could be considered a normal occurrence.

The average chlorophyll a value and range (represented by the vertical line) of the three replicate flasks grown for each solution are shown in Figures 6-11. Figures 6-8 make monthly comparisons of the three salt solutions in straight river water and no sewage effluent (RW), 2 parts river water and 1 part sewage effluent (2 RW: 1 SE), and 1 part river water and 2 parts sewage effluent (1 RW: 2 SE), respectively. Figures 9-11 show the algal growth responses of the three RW:SE combinations as they change with time and are mixed with no salt, 5,000 mg Cl/l and 10,000 mg Cl/l, respectively.

Table 2. Chlorophyll a values (mg/m^3) of 3 replicate flasks obtained from the 9 experimental solutions for each month sampled (November 1976-March 1977).

Cl Conc	RW:SE	Nov	Dec	Jan	Feb	Mar
0 mg/l	3:0	42.63 44.06 39.92	98.90 149.15* 100.50	47.45 69.68 178.20	45.98 38.49 56.13	95.43 87.67 81.08
5,000	3:0	94.09 104.25 81.26	538.88 206.89 391.50	259.01 275.85 237.36	565.07 468.31 395.60	523.24 467.24 442.29
10,000	3:0	49.90 14.70 30.29	151.56 137.84 90.62	166.66 145.50 139.17	160.38 138.28 118.15	218.21 201.10 180.25
0	2:1	411.64 292.64 392.71	319.69 131.20 195.31	436.23 310.07 280.66	157.31 184.44 147.28	423.40 388.12 347.49
5,000	2:1	877.41 929.94 779.18	871.40 913.10 701.40	736.68 744.16 548.86*	807.51 833.98 751.65	910.42 683.22 599.82
10,000	2:1	144.74 145.50 131.20	430.22 324.77 259.46	294.03 296.52 272.56	314.34* 254.83 257.54	216.74 291.09 236.65
0	1:2	170.09 122.96 236.69	296.52 312.34 343.04	544.40 555.98 364.86	208.14 205.82 288.55*	701.66 684.64 811.52
5,000	1:2	1090.41 1125.07 1414.55	1639.44* 1168.99 1167.57	1318.68 1206.19 1230.12	1337.04 1331.69 1035.39*	1334.36 1356.28 718.50*
10,000	1:2	171.52 176.42 211.97	346.15 193.88 255.49	256.34 181.23 159.04	288.15 176.06 184.44	98.37 125.68 81.26

*denotes statistically significant outlier at the 0.05 level.

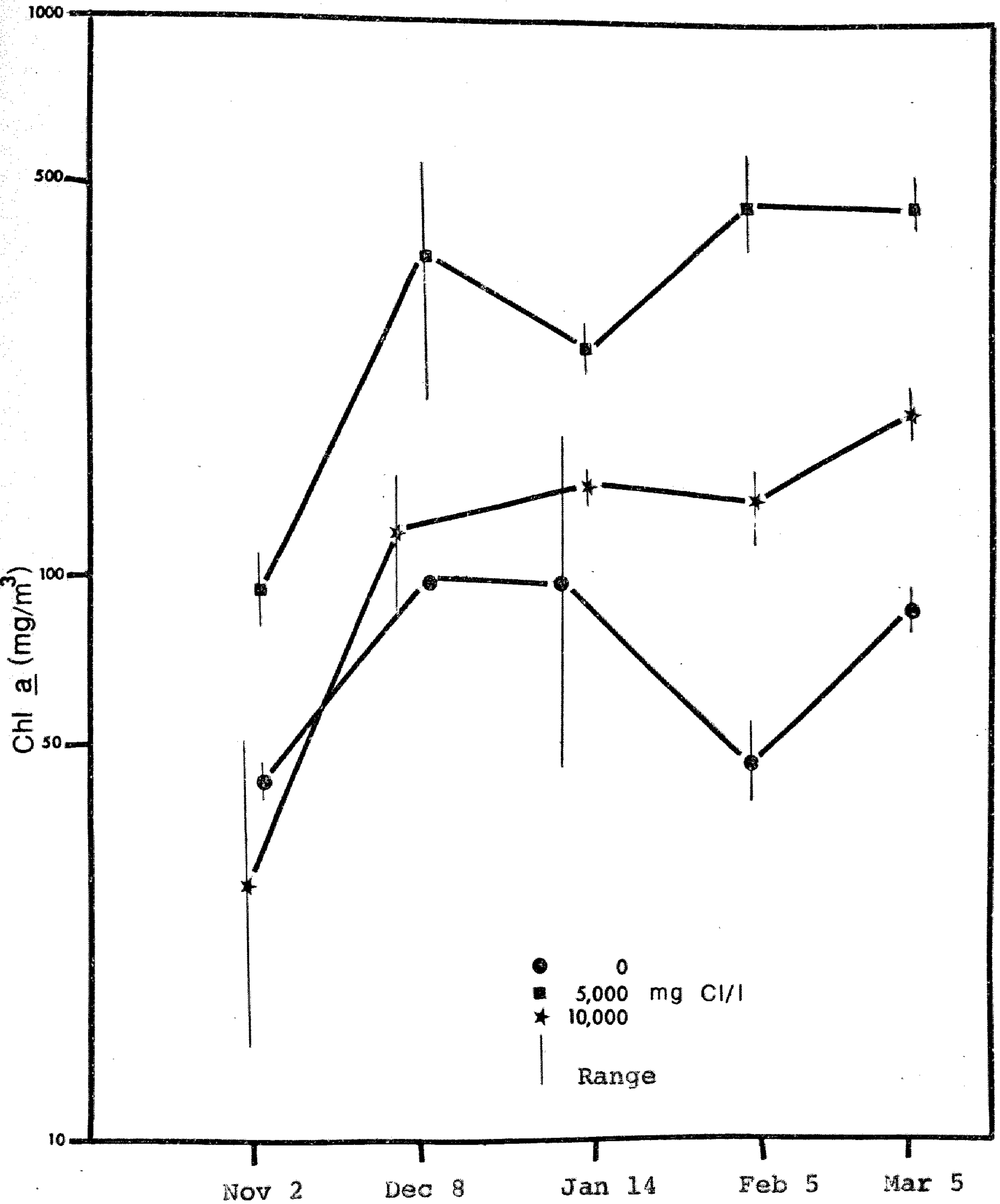

Figure 6. Average chlorophyll a values (mg/m^3) with range of 3 replicate flasks obtained from varying salt concentrations in straight river water (no sewage effluent) over time (monthly samples).

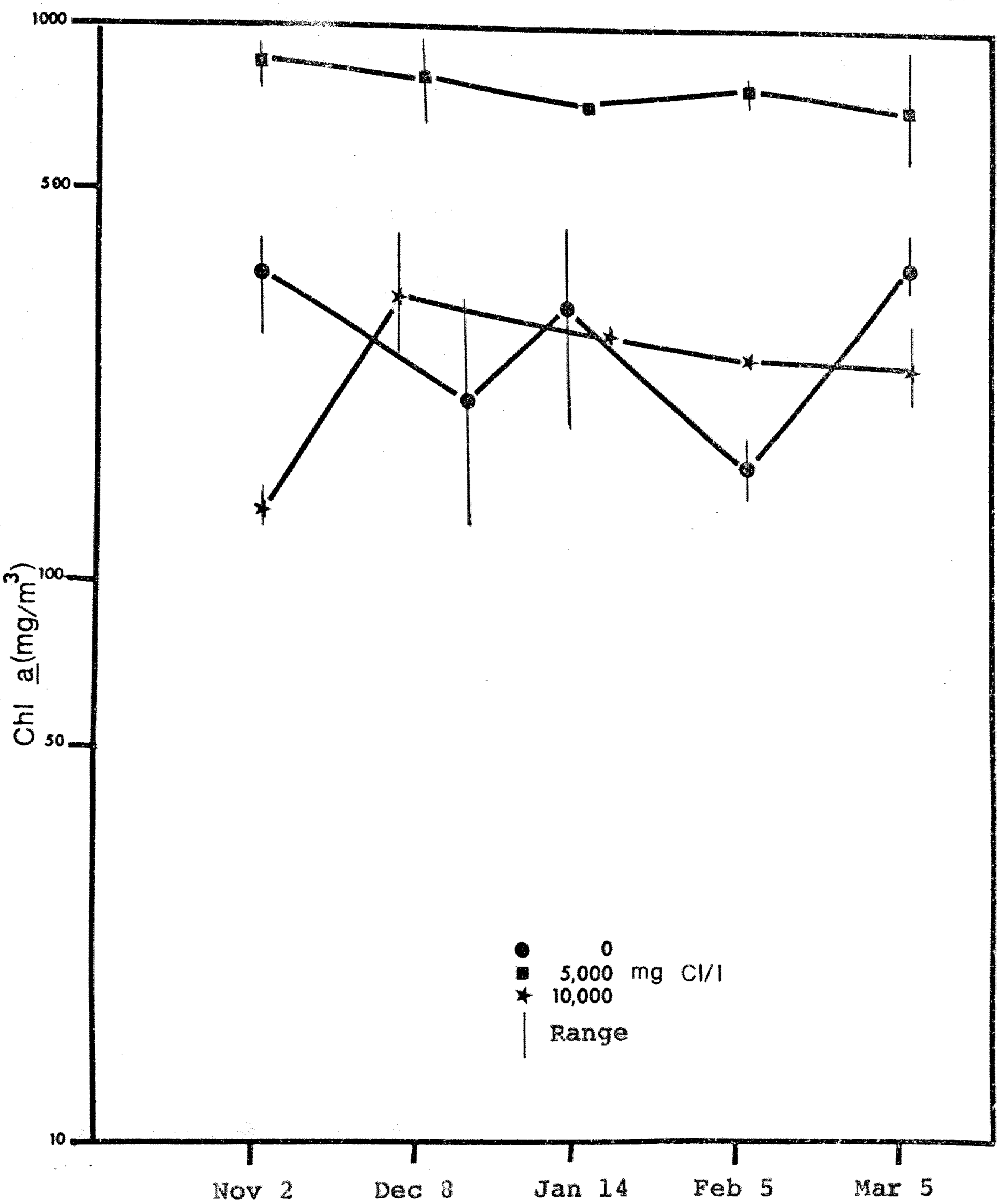

Figure 7. Average chlorophyll a values (mg/m^3) with range of 3 replicate flasks obtained from varying salt concentrations in 2 parts river water to 1 part sewage effluent over time (monthly samples).

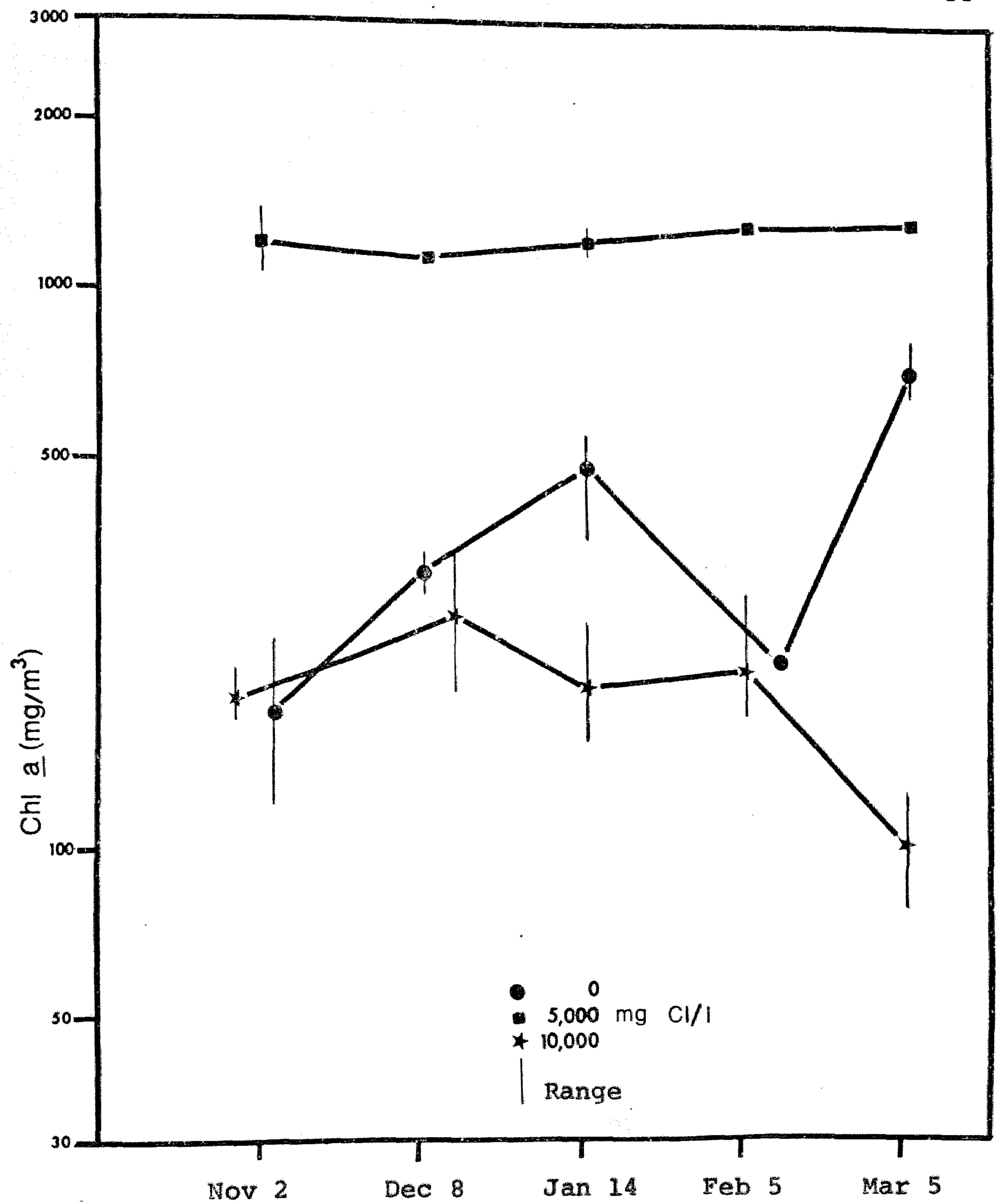

Figure 8. Average chlorophyll a values (mg/m^3) with range of 3 replicate flasks obtained from varying salt concentrations in 1 part river water to 2 parts sewage effluent over time (monthly samples).

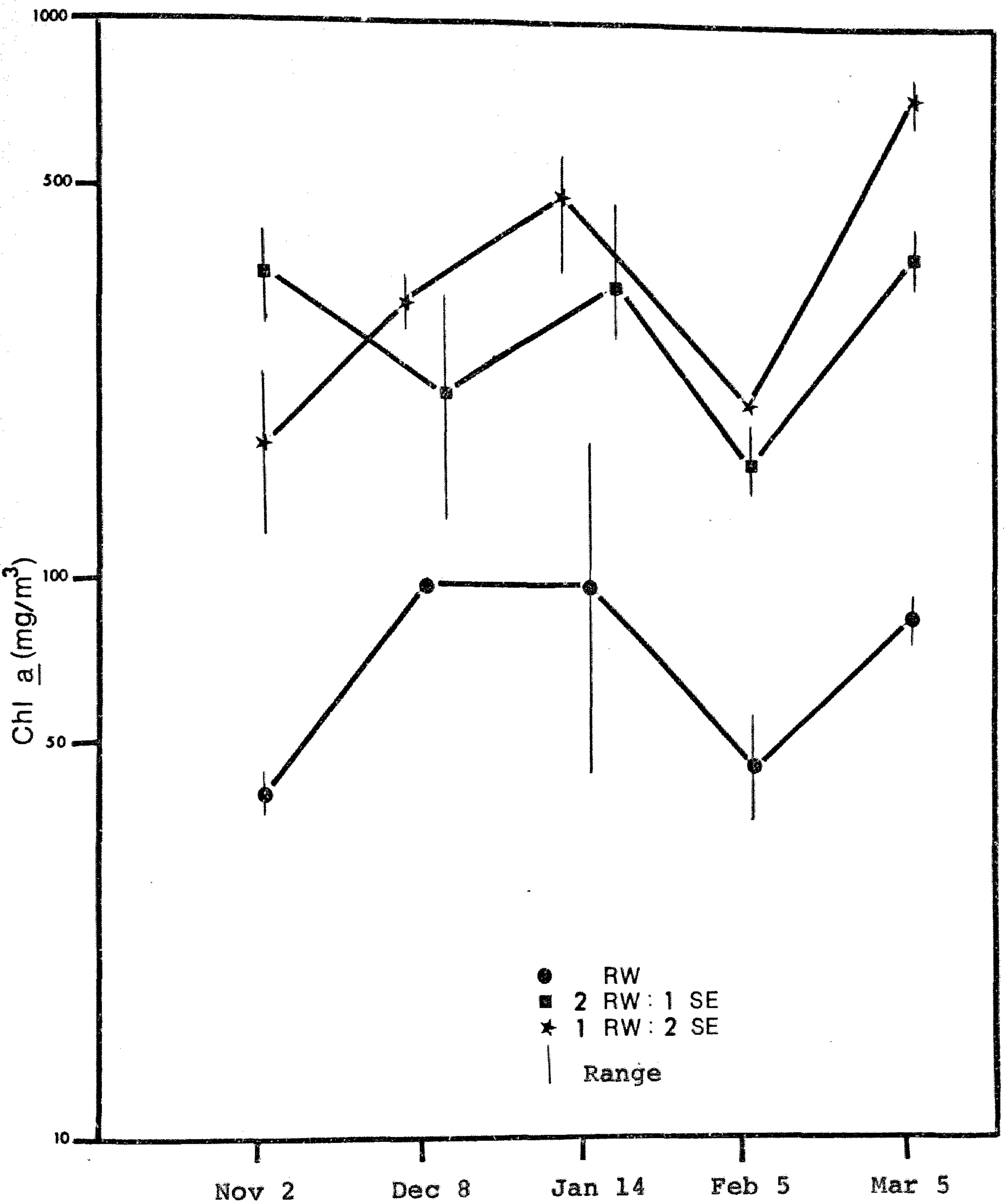

Figure 9. Average chlorophyll a values (mg/m^3) with range of 3 replicate flasks obtained from varying sewage concentrations in straight river water (no salt) over time (monthly samples).

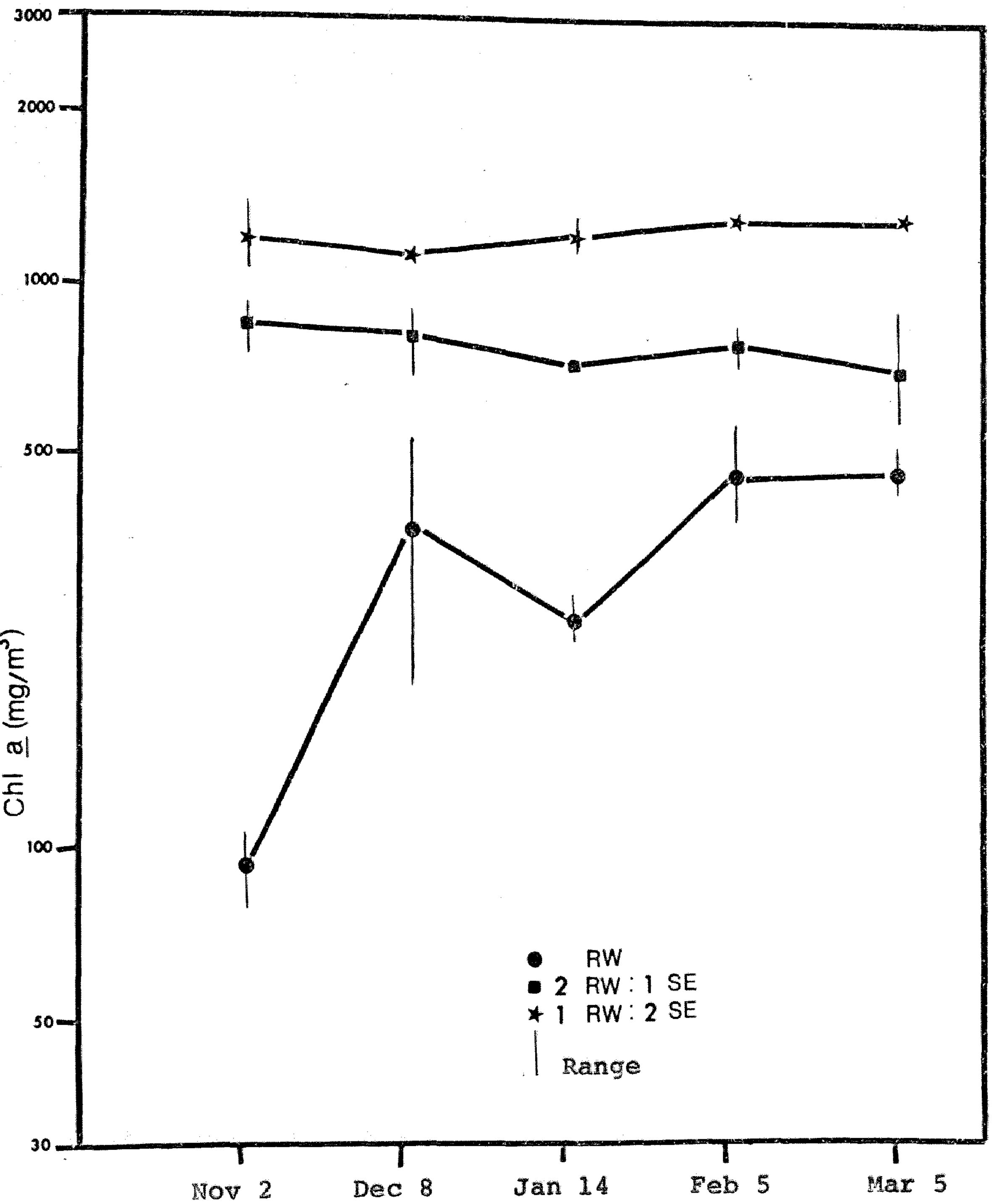

Figure 10. Average chlorophyll a values (mg/m^3) with range of 3 replicate flasks obtained from varying sewage concentrations in approximately 5,000 mg/l of chloride over time (monthly samples).

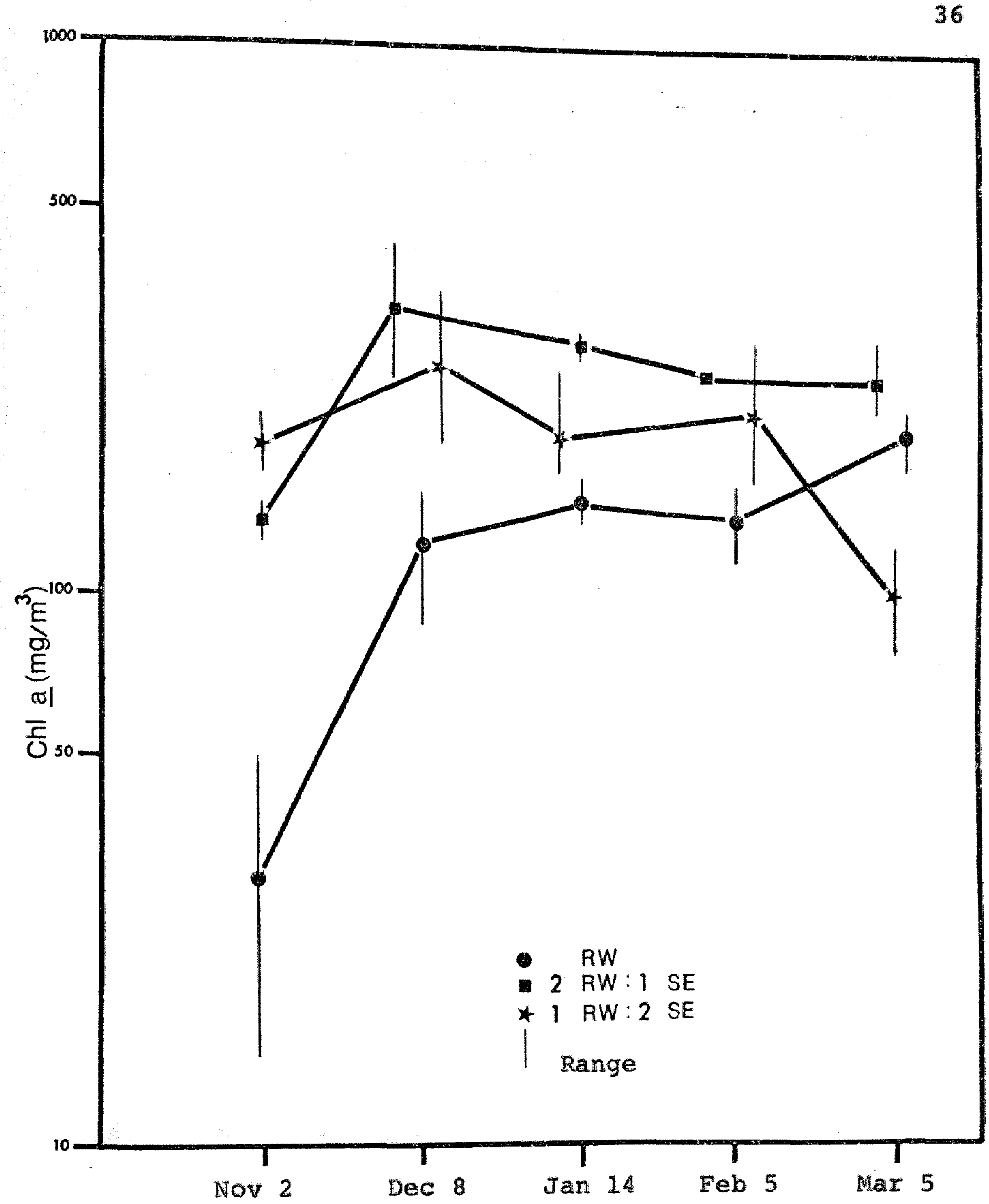

Figure 11. Average chlorophyll a values (mg/m^3) with range of 3 replicate flasks obtained from varying sewage concentrations in approximately 10,000 mg/l of chloride over time (monthly samples).

By examining Table 2 and Figures 6-8, it can be seen that the addition of 5,000 mg Cl/l had a definite enriching effect. The algal growth response is much greater than with no salt or 10,000 mg Cl/l in any concentration of sewage. Even with no SE present (Figure 6), 10,000 mg Cl/l had a greater stimulating effect on the alga. In the two combinations of RW and SE (Figure 7 and 8), there were varied responses with no salt and 10,000 mg Cl/l between months. Figure 6 also shows that straight RW gave lower chlorophyll a values for the November samples than for any other month in all three mixtures of salt.

A check of Table 2 and Figures 9-11 reveals that straight RW has a much lower response than 2 RW: 1 SE or 1 RW: 2 SE in all three salt combinations (except for March, 1 RW: 2 SE, and 10,000 mg Cl/l, see Figure 8). Another thing to note is the difference in response between 1 RW: 2 SE, 2 RW: 1 SE, and straight RW in 5,000 mg Cl/l (Figure 10). There is no overlap between the ranges of any mixture. The response is approximately the same with no salt present (Figure 9), however, some overlap occurs between the December and January samples. Figure 11 shows the 2 RW: 1 SE solution to have the greatest chlorophyll a values though range overlap is common. It also shows lower growth responses for collections from November than those from December-March for the three RW:SE solutions and 10,000 mg Cl/l. One exception is 1 RW: 2 SE in which the March value is significantly less than November.

Table 3 summarizes the analysis of a 3 x 3 factorial design (Bruning and Kintz, 1977) performed to determine the effects of the three variables (salt, sewage and time). A statistically significant difference at the 0.001 level occurred between: (1) the three salt solutions, (2) the three sewage concentrations, (3) interaction between the salt and sewage mixtures, (4) salt and time, and (5) salt and sewage over time. There was a significant difference at the 0.005 level between months (time). Sewage was essentially constant in its composition over time as this interaction showed no significance.

A Newman-Keuls' multiple range test (Bruning and Kintz, 1977) was performed on each group that showed statistical significance to determine where the difference was located. The three combinations of sewage differed significantly from each other. Because there was no variation in algal response to the sewage over time, all the values for each month could be grouped together within each RW:SE mixture, making the analysis much simpler. The Newman-Keuls' multiple range test, using the time variable, revealed that November values differed significantly from December, January, and March values. No significant differences were found between the remaining monthly combinations (November and February, December and March, January and December, January and March, February and December, February and January, and

Table 3. Analysis of the effects of the 3 variables (salt, sewage effluent, and time) in combination with each other.

Source	SS	df	MS	F	p
Total	18,552,028.85	134	----	----	----
Salt	9,441,240.68	2	4,720,620.34	481.14	.001
Sewage	4,112,898.13	2	2,056,449.06	209.60	.001
Time	190,702.82	4	47,675.70	4.85	.005
Salt x Sewage	2,781,599.56	4	695,399.89	70.87	.001
Salt x Time	378,689.08	8	47,336.13	4.82	.001
Sewage x Time	160,252.07	8	20,031.50	2.04	n.s.
Salt x Sewage x Time	603,639.43	16	37,727.46	3.84	.001
Error	883,007.08	90	9,811.18	----	----

February and March).

The multiple range test with salt as the variable was made more complicated by the fact that the salt combinations varied over time. Each monthly salt concentration had to be tested against every other one making a total of 45 group comparisons. Two major patterns emerged from this analysis. The groups presenting the greatest growth responses (1 RW: 2 SE and 5,000 mg Cl/l) were essentially equal in effect and all significantly different from any other group. The next prominent combination consisted mostly of those groups with 5,000 mg Cl/l and 2 RW: 1 SE. The remaining 31 groups had enough overlap between them that no particular trend could be detected.

The Pearson product-moment correlation analysis (Bruning and Kintz, 1977) was performed pairing the average chlorophyll _a_ values of the 9 experimental solutions for each of the five months samples (Table 2) with the observed or calculated phosphate value of the same solution (Table 4) for a total of 45 pairs. The same procedure was used pairing chlorophyll _a_ values with total nitrite-nitrate amounts. The _r_ values calculated for phosphate and total nitrite-nitrate were +.423 and +.411, respectively, with each significant at the 0.01 level. Further computation provided _z_ values greater than ±1.96 ($P\text{-}PO_4$=2.805, $NO_2\text{-}NO_3$= 2.726), confirming that the _r_ values were significant at the 0.05 level using a two-tailed test. Thus, results of the

Table 4. Phosphate, Total Nitrite-Nitrate, and Chloride values for the experimental solutions, November 1976-March 1977. ($P\text{-}PO_4$ - mg/l; N - mg/l; Cl - mg/l)

		RW	SE	RW:SE 2:1	RW:SE 1:2	Actual Chloride Values RW 5,000	RW 10,000	2 RW:1 SE 5,000	2 RW:1 SE 10,000	1 RW:2 SE 5,000	1 RW:2 SE 10,000
Nov 2	P	0.04	1.25	0.47*	0.85*						
	N	0.52	4.52	1.85*	3.18*						
	Cl	58	82	66	74	5,000	5,100	4,900	9,600	4,700	9,700
Dec 8	P	0.03	1.13	0.39*	0.76*						
	N	1.70	3.34	2.23*	2.78*						
	Cl	56	100	71	85	4,700	10,200	4,700	10,100	4,600	9,600
Jan 14	P	0.03	0.75	0.27*	0.51*						
	N	1.26	2.23	1.59*	1.91*						
	Cl	76	118	90	104	5,400	10,900	5,400	10,600	5,300	10,600
Feb 5	P	0.03	0.55	0.20*	0.38*						
	N	1.38	2.70	1.82*	2.26*						
	Cl	102	140	115	127	5,100	9,800	5,000	9,800	5,100	9,300
Mar 5	P	0.13	0.88	0.38*	0.63*						
	N	1.47	3.34	2.09*	2.71*						
	Cl	46	102	65	83	5,100	10,400	5,100	10,300	5,000	10,400

*denotes calculated values based on observed concentrations in RW and SE.

Pearson product-moment correlation analysis indicated a positive relationship between algal growth and both phosphate and total nitrite-nitrate values.

DISCUSSION

The algal assay methods were modified slightly from the recommended procedure (EPA, 1971a). Most algal assays employ continuous fluorescent illumination with a temperature of 24° ± 2°C (Skulberg, 1967; Miller and Maloney, 1971; Emery et al., 1973; McDonald and Clesceri, 1973; Ferris et al., 1974; Schelske et al., 1974; Greene et al., 1975; and Shiroyama et al., 1975). However, continuous illumination caused an increase in incubator temperature to undesirable levels. Therefore, an 18-hour day (at 97.5 foot-candles) with 6 hours of darkness was implemented which maintained the incubator temperature at a more constant and favorable 26° ± 2°C. It is doubtful that the compromises made would have a serious effect on the general results should the procedure be repeated.

The results of this study show that the addition of both secondary sewage effluent and deicing salt to Des Moines River water has a growth-enhancing effect on the test alga, *Selenastrum capricornutum*. The stronger sewage concentrations gave a greater response which is in agreement with previous workers (Megard, 1969; Miller and Maloney, 1971; and Ferris et al., 1974).

The addition of salt without any sewage effluent (Figure 6) also produced a growth-enhancing effect, with 5,000 mg/l of chloride producing a greater response than 10,000 mg/l. There are at least two plausible explanations for this effect. The salt used was a mined rock salt and, therefore, not 100% pure sodium chloride. It is conceivable that when the salt dissociated into solution, it released small amounts of trace nutrients which stimulated algal growth. Secondly, the possibility exists that the concentration of rock salt either inhibited or neutralized the effect of some toxicant present in the river water allowing for a more luxuriant growth. At 5,000 mg Cl/l, this counteraction effect was more noticeable. The 10,000 mg Cl/l concentration may have become somewhat toxic itself, thereby lowering the response. However, it was not totally inhibitory as the growth was still greater than river water with no salt present.

The statistical tests showed a significant difference in algal response to the interaction between salt and time while none was reported between sewage and time (Table 3). Salt was essentially a controlled variable even though concentrations were not quite constant (Table 4). The implication is that river water composition fluctuated which was confirmed by nutrient analysis.

The growth response of algae to straight river water (no chloride added) had its lowest value in November, with

February only slightly greater (Figure 6). The Newman-Keuls' multiple range test confirmed this by indicating November and February samples to be similar but that both differed significantly from December, January, and March collections. Nutrient assays (Table 4) showed phosphate levels in river water to be at their lowest in February, though November concentrations were approximately equal to the average for the five months. Natural chloride values were higher in river water in February than in any other month. When the data were subjected to a regression analysis, a positive correlation between both phosphate ($\underline{r}$=+.423) and total nitrite-nitrate ($\underline{r}$=+.411) values and algal growth was indicated with $\underline{r}$ values significant at the 0.01 level.

It may be possible that the combination of low phosphate and high chloride values inhibited algal growth in the February sample. This would correspond to the results of Vosjan and Siezen (1968) and Chimiklis and Karlander (1973) who concluded that high salt concentrations decreased freshwater algal growth. The fact that all three salt concentrations, with no sewage present (Figure 6), received the poorest response with the November river water sample indicates that some other unmeasured factor depressed growth since nutrient levels were in accordance with, but lower than, those found to be growth-limiting by Shiroyama et al. (1975). It is interesting to note that while the algal response to 5,000 mg/l of chloride stayed relatively constant from February to March, both 0 mg Cl/l and 10,000 mg Cl/l showed increased algal growth. This suggests a possible direct influence by the March concentrations of phosphate and/or total nitrite-nitrate which were both greater than February values (Table 4).

It has been reported that nutrients (phosphorus and nitrogen) are not limiting to phytoplankton growth in Iowa's surface waters (Iowa Department of Environmental Quality, 1975; Jones and Bachmann, 1975; and Kilkus et al., 1975). The chemical analyses performed for five months on the samples in this study indicated that while neither phosphorus nor nitrogen were present in large quantities, they could influence phytoplankton populations, as shown by the positive correlation between nutrient concentrations and algal response and increased growth with sewage effluent additions. The highest phosphate value in March river water corresponds to the most algal growth with salt added (Figure 6). The largest chlorophyll a values for growth in straight river water occurred in December samples which contained the greatest total nitrite-nitrate levels. The wide range of the January growth response in straight river water is the result of uneven growth among the three replicate flasks (Table 2, Figures 6 and 9). If the largest value is omitted, the average chlorophyll a concentration would approximate 60 mg/m^3 and lower the January figure. This parallels a plot of the nitrogen concentration in the monthly river collections.

Phytoplankton growth in the Des Moines River appears to be influenced by the addition of both nitrogen and phosphorus compounds. It is possible that nutrients present in the rock salt were solubilized by dissociation of the mineral resulting in stimulated growth. Treated March river samples, with the highest phosphate concentrations, gave the greatest responses for the two salt solutions. Reduced growth in the 10,000 mg/l concentration of chloride is apparently due to the slightly inhibitory effect of the high level of salt.

Figure 9 shows that the algal response in river water and sewage mixtures closely follows that of the highest nutrient concentrations (Table 4). This corresponds to work published by Skulberg (1967), Miller and Maloney (1971), and Shiroyama et al. (1975). By following the graph of 1 RW: 2 SE, it is noted that the growth response exceeds the solutions of straight river water and 2 RW: 1 SE except for November. The Newman-Keuls' multiple range test indicated that the November values were significantly different from December, January, and March values. Although the greatest amounts of both phosphate and total nitrite-nitrate occurred in November sewage, the growth response of 1 RW: 2 SE is less than that of 2 RW: 1 SE for the month. Shiroyama et al. (1975) found a definite linear relationship between maximum algal yield (mg/l) and both phosphorus concentrations up to approximately 0.300 mg/l and nitrogen concentrations up to about 2.10 mg/l. Above these levels, other nutrients became limiting. This appears to be the case with the November growth response of 1 RW: 2 SE as both the phosphorus and nitrogen values exceed the published values.

The growth-enhancing effect of the addition of both deicing salts and nutrients in any algal assay conducted under laboratory conditions, such as this one, would be difficult to extrapolate to natural environmental conditions unless field experiments were performed. The use of deicing chemicals promotes snowmelt which increases runoff from urban areas. This runoff includes not only higher salt concentrations, but also pollutants which increase nutrient, suspended solids, and turbidity levels, and its stimulatory effect has already been documented (Sartor et al., 1974). Deicing runoff occurs during months least favorable to algal growth because of temperature and light limitations, thus increases in phytoplankton populations may not be readily recognized. However, the possibility exists that, given the proper circumstances (i.e., during spring months with rising temperature, increased snowmelt runoff from urban and rural areas which adds to nutrient levels, and longer days), the combination of deicing salts and sewage effluents could result in an algal bloom. A similar situation might arise if salt values are increased during summer months because of increased salt in groundwater.

Present salting programs in the Des Moines area do not stress the bare pavement policy that was popular in the 1960's. Decisions about the amount of salt used rest on close cooperation between local weather services and the Public Works Administration and depend on many factors (type of storm, time and day of week). As a result, the salting policy is one of restraint and judgment based on experience. During a winter such as that of 1976-77, no problem appears to exist regarding deicing salt pollution. However, many small storms would necessitate a substantial increase in salt use. It is probable, though, that such a volume of snowmelt would occur so as to dilute the chloride concentrations to safe levels. Obviously, weather is an important factor in any situation.

While Des Moines is not in a position to be very concerned about deicing runoff, many other large metropolitan centers are. The bare pavement policy is often still followed due to strong public pressure to keep winter roads clear for commuters. The highest chloride values in runoff and surface waters have been recorded near heavily populated areas such as Milwaukee, Chicago, Ann Arbor, and Rochester. Many have been in excess of 10,000 mg/l of chloride with some values approaching 25,000 mg/l. Studies published by Judd (1970), Bubeck et al. (1971), and Diment et al. (1973) have shown significant effects of salt on the physical parameters of lakes (i.e., preventing or delaying overturn).

However, a limited amount of data have been published concerning the effects on running waters or the aquatic biota. At these high concentrations, it is likely that inhibition or some other toxic effect occurs rather than stimulation.

It is increasingly important that engineers regard the environmental effects of road building and maintenance as a major consideration in designing public highways. The development of the Environmental Protection Agency and other regulatory agencies in recent years reflects the growing awareness of our fragile natural resources and the need to preserve and maintain them.

Compliance with Federal regulations by point source dischargers should significantly improve Iowa's water quality. The Iowa DEQ (1975) urged implementation of a non-point source control program to reduce the levels of turbidity, nutrients, and toxic substances. Additional study is also needed to determine the impact of salt on aquatic life.

This topic could be pursued in a number of directions. Data collected from a year-round study or over several years would be most valuable in determining present chloride levels and serving as a basis from which to predict future trends. Increasing the number of sampling sites along the river, both upstream and downstream from Des Moines, might give added insight to the influence of street deicing in urban areas on the aquatic environment. The use of particular nutrient spikes in addition to various salt levels could show which interactions are most favorable or inhibitory to algal growth. Another study involving a wider range of salt concentrations would also be useful in determining algal reactions to deicing chemicals. Experiments could be conducted with reagent grade NaCl to compare results with those from the use of deicing salt in an effort to determine the influence of possible trace elements released by the latter. Additional extensions of this research might employ indigenous algal species found in local surface waters. An *in situ* study would provide meaningful data regarding algal response to chemical changes in their environment under the actual physical conditions.

It has been shown that the addition of deicing salt and sewage effluent to Des Moines River water results in increased algal growth. High concentrations of chloride (10,000 mg/l) produced less of a stimulatory effect than 5,000 mg/l, but more than straight river water. The growth response to the mixture of 1 RW: 2 SE with 5,000 mg Cl/l was significantly greater than 2 RW: 1 SE with 5,000 mg Cl/l. Both effects were significantly different from those caused by 0 mg Cl/l and 10,000 mg Cl/l depressed or cancelled out the influence of nutrient additions.

SUMMARY

1. *Selenastrum capricornutum* was grown in an algal assay which combined various concentrations of deicing salt

and sewage effluent diluted with Des Moines River water. Growth response was measured with a chlorophyll assay.

2. Monthly river and sewage samples were taken from November 1976-March 1977. Nutrient levels were determined by chemical analyses.
3. Stronger sewage concentrations gave greater responses with and without salt, except for 10,000 mg Cl/l where the lower sewage level had the highest chlorophyll values.
4. The addition of 5,000 mg Cl/l was stimulating in all combinations of river water and sewage effluent tested.
5. Although 10,000 mg Cl/l was not inhibitory, it may be slightly toxic since the growth response was less than the lower salt concentration and more like that of straight river water.
6. November samples gave the lowest chlorophyll values while those from March usually had the greatest which corresponded to nutrient values.
7. Further study should include a year-round survey with more sampling sites and frequent collections to determine present chloride levels and serve as a basis for predicting future trends.
8. Both sewage effluent and deicing salt had a growth-enhancing effect on the test alga in most combinations.

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